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EGFR expression is associated with groin node metastases in vulvar cancer, but does not improve their prediction

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Abstract

Objectives. High morbidity of elective inguinofemoral lymphadenectomy in early stage vulvar cancer patients urges the need for defining a group of low-risk patients in whom inguinofemoral lymphadenectomy can be safely omitted. Aim of the study was to evaluate whether in addition to 'classic' clinicopathological factors determination of EGFR expression in vulvar cancer can be helpful in defining such a 'low-risk' group.

Methods. Formalin-fixed paraffin-embedded tumor tissue samples of 197 surgically treated T1/2 patients were collected in a Tissue Micro Array (TMA). On this TMA, immunohistochemistry for EGFR was performed. Logistic regression analyses were performed including histopathological characteristics with the presence of nodal metastases as outcome. A predictive model was constructed, and absolute risks were calculated.

Results. EGFR expression was present in 68% of the vulvar tumors and related to the presence of lymph node metastases (OR 2.12, 95% CI 1.09–4.10). Our predictive model with only clinicopathological factors was able to define a group of patients with a likelihood of absence of lymph node metastases of 13% (95% CI 5–36), which could be decreased to 6% (95% CI 0–29) after inclusion of EGFR expression ($p=0.07$).

Conclusions. EGFR expression is present in the majority of vulvar tumors and is associated with groin node metastases in vulvar cancer. Current classic clinicopathological predictive factors for inguinofemoral lymph node metastases with or without EGFR analysis are not strong enough for identification of "sufficiently low" risk T1/2 vulvar cancer patients. Our predictive model approach however is excellent for evaluation of new cell biological parameters, associated with clinical outcome.
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Keywords: Vulvar cancer; Lymphatic metastases; Epidermal growth factor receptor

Introduction

Carcinoma of the vulva is a rare disease which predominantly affects elderly women. The most common histological type is squamous cell carcinoma which accounts for 90% of all vulvar carcinomas [1]. Standard treatment for squamous cell carcinoma of the vulva is wide local excision with elective uni- or bilateral superficial and deep inguinofemoral lymphadenectomy via separate incisions. Although effective with respect to cure, the morbidity of this treatment is high and especially related to the lymphadenectomy with frequent wound breakdown, infections, and lymphedema of the legs as major complications [2,3].

About 20–30% of patients with early stage squamous cell carcinoma of the vulva (T1 (≤2 cm) or T2 (>2 cm) tumor, without suspicious groins) will have inguinofemoral lymph node metastases. In retrospect, 70–80% of these patients will therefore probably not benefit from complete inguinofemoral lymphadenectomy but are at risk for its major complications.

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Moreover, it has to be kept in mind that missing inguinofemoral lymph node metastases may have major impact for the patients because of the high mortality associated with groin recurrences, especially in an undissected groin [4]. In the light of these considerations, gynecologic oncologists throughout the world regard an elective inguinofemoral lymphadenectomy as standard of care in early stage vulvar cancer patients with depth of invasion >1 mm.

To obviate the morbidity of elective inguinofemoral lymphadenectomy, while maintaining a very low number of groin recurrences, the demands for non- or minimally invasive techniques for exclusion of inguinofemoral lymph node metastases are extremely high. In a recent review [5], it was shown that currently available non-invasive diagnostic tests (palpation, ultrasound (with or without FNA cytology), CT, MRI, PET) are not able to exclude inguinofemoral lymph node metastases with a certainty that is high enough to safely omit complete inguinofemoral lymphadenectomy, while the safety of the sentinel node technique is currently being evaluated in phase II studies [6–8]. Studies on different histopathogical parameters of the primary vulvar tumor showed a very low (<1%) risk for inguinofemoral metastases in tumors with a depth of invasion less than 1 mm [9–13]. No other 'classic' histopathological parameters are able to define comparable "low-risk groups" of patients with invasive squamous cell carcinoma of the vulva. In addition, a variety of cell biological parameters, more or less associated with metastatic behavior of the primary tumor, have been evaluated for their predictive value, but overall these data are inconclusive and conflicting, especially because the number of patients analyzed in these studies is often too small.

So far, the only parameter with potentially high enough negative predictive value to exclude lymph node metastases in patients with vulvar carcinoma was the absence of epidermal growth factor receptor (EGFR) overexpression. EGFR activation plays a key role in cell adhesion, cell locomotion, cell survival, invasion, and angiogenesis, which results in modulation of tumor progression, e.g. metastases [14].

In this study, we analyzed the relation between expression of EGFR with inguinofemoral lymph node metastases in a large group of patients with early stage squamous cell carcinoma of the vulva. As the focus for the clinician is to find a way to avoid an elective inguinofemoral lymphadenectomy, we then constructed a predictive model including classic clinicopathological and immunostaining parameters to identify a group of patients with a low likelihood of presence of inguinofemoral lymph node metastases.

Patients and methods

Patients

Since 1984, clinicopathological and follow-up data of all patients referred to the Department of Gynecologic Oncology of the University Medical Center Groningen, The Netherlands are prospectively collected during standard treatment and follow-up and stored in a computerized registration database, which is managed in accordance with the hospitals regulations. For the current study, all consecutive squamous cell vulvar cancer patients with T1–2 tumors treated from March 1984 until January 2000 were selected. Patients who did not undergo inguinofemoral lymphadenectomy were excluded (n=25); 7 because of FIGO stage IA disease and 18 because of bad general health. Until 1993, standard treatment consisted of radical vulvectomy with en bloc uni- or bilateral inguinofemoral lymphadenectomy. Since 1993, this treatment was modified to wide local excision with uni- or bilateral inguinofemoral lymphadenectomy via separate incisions. Staging was performed according to the surgicopathologic FIGO classification [15] and the AJCC TNM classification [16].

IRB approval

For the present study, all relevant data were retrieved from our previously mentioned, larger, computerized database into a separate, anonymous database. In this separate, password protected database, patient identity was protected by study-specific, unique patient numbers, which codes were only known to two dedicated datamanagers, who also have daily responsibility for the larger database. In case of uncertainties with respect to clinicopathologic and follow-up data, the larger databases could only be checked through the datamanagers, thereby ascertaining the protection of patients' identity. Due to these procedures according to Dutch law, no further patient or IRB approval was needed.

Histology

Results of the histological diagnosis of the biopsy and surgical specimen were taken together. Biopsies examined at regional pathology laboratories were requested for review. The tumors were examined for tumor thickness, degree of differentiation, vascular invasion, and multifocality. Using a calibrated eyepiece micrometer, tumor thickness was measured from the tumor surface or, in the case of superficial keratosis, from the level of the stratum granulosum downward to the deepest point of the tumor; the thickness of the keratin layer, if present, was therefore neglected. The degree of differentiation in the most prominent part of the tumor was assessed according to the criteria described elsewhere [17]. Capillary-lymphatic infiltration was defined as the presence of tumor emboli in endothelial-lined spaces. The inguinofemoral lymph nodes from each side were examined (one section per cm node) for the presence of intranodal or extranodal metastatic tumor growth.

Immunohistochemistry

Formalin-fixed paraffin-embedded tumor tissue samples from all patients were available for constructing Tissue Micro Arrays (TMAs). Morphologically representative areas of the primary tumor were marked on hematoxylin- and eosin-stained sections. Areas of necrosis or severe leukocyte infiltration were avoided. Three cores of 0.6 mm in diameter were taken from the marked areas out of the corresponding tissue block and were placed in pre-defined array locations in a recipient blank paraffin block, using a precision instrument (Beecher Instruments, Silver Spring, Maryland). After inserting the cores, the blocks were placed in an oven of 37°C for 30 min in order to attach the cores to the surrounding paraffin. In total, three arrays were constructed, each containing three cores per tumor. Cores of histologically normal vulvar tissue and vulvar cancer tissue originating from the same donor blocks were incorporated in all three arrays to serve as internal control for intra-run variability and to help to orientate oneself in the TMA. Sections of 4 μm were cut from the arrays and were transferred to adhesive coated slides. Immunohistochemistry for EGFR was performed on these TMAs with primary mouse anti-human EGFR monoclonal antibody (clone 31G7, Zymed, #28-005). Slides were cleared in xylene and rehydrated through a graded ethanol series in distilled water. They were subjected to antigen retrieval using a 0.1% Trypsin (0.1% Calcium Chloride) solution at 37°C for 10 min. Immunostaining was performed using the DAKO autostainer. Diaminobenzidine (DAB) was used as chromogen to visualize the antibody. The nuclei were counterstained in Mayer's hematoxylin, dehydrated in graded ethanol, dried and coverslipped. For EGFR staining, sections from an ovarian cancer specimen with well established EGFR overexpression were used as

Table 1
Patient and disease characteristics related to the EGFR (*n* (%); χ^2 test; *p* value)

Characteristics	EGFR (moderate/strong) positive	χ^2 test
Age		
Under age 73	59 (60.2)	$\chi^2=4.75$
73 and over	74 (74.7)	$p=0.03$
T-status		
T1 (maximum diameter ≤2 cm)	27 (58.7)	$\chi^2=2.13$
T2 (maximum diameter >2 cm)	106 (70.2)	$p=0.15$
Depth of infiltration		
≤5 mm	42 (54.5)	$\chi^2=10.3$
>5 mm	91 (76.5)	$p=0.001$
Grade of differentiation		
Good	51 (65.4)	
Moderate	69 (75.8)	$\chi^2=8.70$
Poor	13 (46.4)	$p=0.01$
Vascular invasion		
No	111 (65.7)	$\chi^2=1.82$
Yes	54 (78.6)	$p=0.18$

positive controls. Isotype IgG antibodies replacing the primary antibody served as negative control.

The staining intensity for EGFR observed in each core was scored on a four-point scale for EGFR (0=negative, 1=weakly positive, 2=positive, 3=strongly positive). Scoring was independently performed by two of the authors (KA ten Hoor and DJ van der Veen), and a concordance of more than 95% was found. The discordant cases were reviewed, and scores were reassigned on consensus of opinion. For our statistical analyses, we used the average intensity score of three cores.

Statistics

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 12.01. To determine whether single predictive factors differed significantly between patients with and without nodal metastasis at the moment of diagnosis, odds ratios were estimated by univariate logistic regression with the presence of nodal metastases as dependent variable. Then, a multivariate logistic regression analysis was performed with again the presence of nodal metastasis as dependent variable, including the variables that contributed statistically significantly in the univariate analysis. Based on this, two predictive models were constructed: one with only classic predictive factors, and one with inclusion of EGFR. The values of the β-coefficients of the variables included in the multivariate logistic regression equation were used to generate two scoring formulas, one including classic predictive factors and one including classic predictive factors as well as EGFR. The predicted probability for the presence of nodal metastases in both models was calculated for each patient. In the following, it was verified how many subjects were identified correctly by using different cut off values of the scoring formulas. The observed values according to the value of the scoring formulas were grouped, and the observed numbers of patients in total as well as the observed numbers of patients with nodal metastasis were counted. All tests were two-sided and *P* values of <0.05 were considered significant.

Results

Patients

Between March 1984 and January 2000, 197 patients with primary T1 or T2 squamous cell tumors of the vulva were surgically treated at the University Medical Center Groningen, The Netherlands. Median age was 73 years (range: 34–94). Forty-six (23.4%) patients had a T1 tumor and 151 (76.6%) a T2 tumor. The primary vulvar tumor was unilateral in 111 patients (56.3%) and bilateral in 85 patients (43.2%) (one missing value). Multifocality of the primary tumor was seen in 30 patients (15.2%). One or more inguinofemoral lymph node metastases were present in 71/197 patients (36%). Thirty patients had one positive inguinofemoral lymph node, 19 patients two, and 11 patients three or more positive inguinofemoral lymph nodes. Follow-up data were available for all patients and have been published previously [18], but are not further reported here.

Immunostaining

Positive staining for EGFR was observed in 68% of the tumors. Positive staining for EGFR was associated with a depth of invasion >5 mm ($p=0.001$) and with good to moderate grade of differentiation ($p=0.01$). There was no relation with age, T-status and vascular invasion. Chi-square tests for EGFR staining in relation to different tumor characteristics are shown in Table 1.

Clinicopathological and immunostaining parameters in relation to inguinofemoral lymph node metastases

For an overview of patient and tumor characteristics related to the presence of lymph node metastases and the univariate

Table 2
Patient and disease characteristics total and related to the presence of nodal metastasis at the moment of diagnosis of primary tumor (*N* (%); univariate OR, 95% CI)

Characteristics	Total (*n*=197)	Presence of nodal metastasis (*n*=71)	Univariate OR (95% CI)
Age			
Under age 73	98 (49.7)	32 (45.1)	1
73 and over	99 (50.3)	39 (44.9)	1.34 (0.75–2.40)
T-status			
T1 (maximum diameter ≤2 cm)	46 (23.4)	9 (12.7)	1
T2 (maximum diameter >2 cm)	151 (76.6)	62 (87.3)	2.86 (1.29–6.36)
Depth of infiltration [a]			
≤5 mm	77 (39.1)	17 (23.9)	1
>5 mm	119 (60.4)	54 (76.1)	2.93 (1.53–5.61)
Grade of differentiation			
Good	78 (39.6)	20 (28.2)	1
Moderate	91 (46.2)	36 (50.7)	1.90 (0.98–3.67)
Poor	28 (14.2)	15 (21.1)	3.35 (1.36–8.23)
Vascular invasion			
No	169 (85.8)	58 (81.7)	1
Yes	28 (14.2)	13 (18.3)	1.66 (0.74–3.72)
EGFR			
Negative	64 (32.5)	16 (22.5)	1
(Moderate/strong) positive	133 (67.5)	55 (77.5)	2.12 (1.09–4.10)

[a] Could not be assessed for one patient.

Table 3
Patient and disease characteristics related to the presence of nodal metastasis ($n=71$) at the moment of diagnosis of primary tumor: a model excluding and a model including EGFR (multivariate OR, 95% CI)

Characteristics	A model excluding EGFR	A model including EGFR
T-status		
T2 (maximum diameter >2 cm)	1.81 (0.76–4.30)	1.75 (0.73–4.20)
Depth of infiltration		
>5 mm	2.53 (1.25–5.01)	2.26 (1.09–4.62)
Grade of differentiation		
Moderate	1.81 (0.91–3.60)	1.75 (0.88–3.50)
Poor	3.33 (1.30–8.53)	3.94 (1.48–10.51)
EGFR		
Positive	–	1.95 (0.93–4.08)

odds ratios by logistic regression, see Table 2. T-status (OR 2.86, 95% CI 1.29–6.36), depth of infiltration (OR 2.93, 95% CI 1.53–5.61), grade of differentiation (good versus poor: OR 3.35, 95% CI 1.36–8.23), and EGFR status (OR 2.12, 95% CI 1.09–4.10) were all associated with the presence of lymph node metastases. The two multivariate logistic regression models, one with and one without EGFR, are shown in Table 3. EGFR did not retain its predictive value in the multivariate analysis. EGFR staining intensity was positive (moderate–strong) in 78/126 (61.9%) of the patients without nodal metastases and in 55/71 (77.5%) of the patients with nodal metastases (OR: 2.1, 95% CI: 1.1–4.1).

A predictive model for inguinofemoral lymph node metastases

The values of the β-coefficients of the tumor characteristics included in the multivariate logistic regression equation were used in the predictive models shown in Tables 3 (without EGFR) and 4 (with EGFR). Our predictive model based on clinicopathological parameters without EGFR allowed identification of T1/2 patients with a likelihood of absence of lymph node metastases of 13% (95% CI 5–36; 30 (15%) patients), which could be decreased to 6% (95% CI 0–29) after inclusion of EGFR expression ($p=0.07$; 17 (9%) patients) (Table 4).

Discussion

Our study shows that EGFR expression is present in the majority of primary squamous cell carcinomas of the vulva and that it is associated with the presence of inguinofemoral lymph node metastases. However, it has no value in the prediction of lymph node metastases.

So far, expression of EGFR in vulvar cancer has been evaluated only to a very limited extent. Berchuck et al. showed in 34 squamous carcinomas of the cervix, vulva and vagina that EGFR expression was moderate to heavy in all malignant cells [19]. Another small study on EGFR expression in vulvar cancer showed progressive increase in EGFR expression from benign vulvar epithelial to primary malignant tissue to metastatic lesions within the same patient. Increased expression of EFGR in the primary vulvar tumor was also significantly associated with lymph node metastases, but its predictive value was not properly addressed [14]. Studies on EGFR expression in other gynecologic malignancies showed that EGFR overexpression is associated with biological aggressiveness and poor prognosis in cervical cancer [20–22]. Multivariate analysis of risk factors for metastatic disease showed that EGFR overexpression in endometrial cancer is an independent predictor for the presence of metastases [23].

Regarding primary squamous cell carcinoma of the vulva, there is a wealth of studies reporting associations between a variety of cell biological parameters and patients characteristics such as presence of lymph node metastases. However, most of these studies give the decision-maker no indication of the absolute number of people who might benefit from adding these biological parameters to routine diagnostics. In the presented study here, after calculating the relative risks for the absence of groin node metastases in vulvar cancer, the absolute risks were calculated for the absence of these metastases. Based on these absolute risks, we were able to

Table 4
Number of patients with different score levels (divided into four groups) in relation to the probability of the absence of nodal metastases at the moment of diagnosis of primary tumor

Scores	A model including EGFR [a]			A model excluding EGFR [b]		
	Number of patients with score[a]	Number (%) without nodal metastasis	95% CI	Number of patients with score[b]	Number (%) without nodal metastasis	95% CI
0–2	16	6 (38%)	0.12–0.70	67	33 (49%)	0.36–0.61
2–4	78	40 (51%)	0.38–0.60	55	32 (58%)	0.43–0.70
4–6	48	33 (69%)	0.53–0.82	44	34 (77%)	0.62–0.88
6–8	37	30 (81%)	0.64–0.90	30	26 (87%)	0.64–0.95
8–9	17	16 (94%)	0.71–1.00	–		

[a] Score=1.75 (if T1-status is present)+2.25 (if depth of infiltration ≤5 mm)+3.94 (if good grade of differentiation is present)+2.26 (if moderate grade of differentiation is present)+1.95 (if EGFR is negative).

[b] Score=1.81 (if T1-status is present)+2.53 (if depth of infiltration ≤5 mm)+3.33 (if good grade of differentiation is present)+1.84 (if moderate grade of differentiation is present).

conclude that, despite the fact that high EGFR expression is associated with inguinofemoral lymph node metastases, its expression analysis allows us to define only a small group of patients (9%) with a likelihood of absence of lymph node metastases of still 6% with a relatively wide 95% CI (0–29). These figures would implicate a false-negative rate for prediction of inguinofemoral lymph node metastases of at least 6%.

The high morbidity of elective inguinofemoral lymphadenectomy in early stage vulvar cancer patients urges the need for defining a group of 'low-risk' patients in whom inguinofemoral lymphadenectomy can be safely omitted. When debating a possible acceptable false-negative rate for sentinel node detection in vulvar cancer the Gynecologic Oncology Group of the European Organisation for Research and Treatment of Cancer (EORTC) decided that in the light of the significant decrease in morbidity by omitting elective lymphadenectomy a maximum increase of groin recurrences (i.e. a false-negative rate) of 6% might be acceptable. Our study showed that based on the currently available clinicopathological data with or without inclusion of EGFR expression analysis we could not identify accurately a cohort of T1/2 patients with such a low likelihood of inguinofemoral lymph node metastases. The upper level of our 95% CI was higher than the required 6%. The predictive model approach that we used for the present study however seems to be quite feasible for the evaluation of the predictive value of future cell biological parameters possibly associated with metastatic behavior.

Currently, no non-invasive technique is available for accurate prediction of inguinofemoral lymph node status. At present, the sentinel node technique seems to be the most promising minimal-invasive technique for defining a group of vulvar cancer patients in whom inguinofemoral lymphadenectomy can be safely omitted. Further research is required in order to find more powerful predictive markers for inguinofemoral lymph node metastases. Our predictive model and the availability of TMAs of early stage vulvar cancer will allow rapid and accurate evaluation of these new markers.

References

[1] Hacker NF. Vulvar cancer. In: Berek JS, Hacker NF, editors. Practical gynecologic oncology. 3rd ed. Baltimore: Williams and Wilkins; 2000. p. 553–96.

[2] Prodratz KC, Symmonds RE, Taylor WF, Williams TJ. Carcinoma of the vulva: analysis of treatment and survival. Obstet Gynecol 1983;61:63–74.

[3] Andersen BL, Hacker NF. Psychosexual adjustment after vulvar surgery. Obstet Gynecol 1983;62:457–62.

[4] Stehman FB, Bundy BN, Ball H, Clarke-Pearson DL. Sites of failure and times to failure in carcinoma of the vulva treated conservatively: a Gynecologic Oncology Group study. Am J Obstet Gynecol 1996;174:1128–32.

[5] Oonk MHM,, Hollema H, de Hullu JA, van der Zee AGJ. Prediction of lymph node metastases in vulvar cancer, a review. Int J Gynaecol Cancer 2006;16:963–71.

[6] De Hullu JA, Hollema H, Piers DA, et al. Sentinel lymph node procedure is highly accurate in squamous cell carcinoma of the vulva. J Clin Oncol 2000;18:2811–6.

[7] Moore RG, DePasquale SE, Steinhoff MM, et al. Sentinel node identification and the ability to detect metastatic tumor to inguinal lymph nodes in squamous cell cancer of the vulva. Gynecol Oncol 2003;89:475–9.

[8] Puig-Tintoré LM, Ordi J, Vidal-Sicart S, et al. Further data on the usefulness of sentinel lymph node identification and ultrastaging in vulvar squamous cell carcinoma. Gynecol Oncol 2003;88:29–34.

[9] Hacker NF, Berek JS, Lagasse LD, Nieberg RK, Leuchter RS. Individualization of treatment for stage I squamous cell vulvar carcinoma. Obstet Gynecol 1984;63:155–62.

[10] Magrina JF, Webb MJ, Gaffey TA, Symmonds RE. Stage I squamous cell cancer of the vulva. Am J Obstet Gynecol 1979;134:453–9.

[11] Ross MJ, Ehrmann RL. Histologic prognosticators in stage I squamous cell carcinoma of the vulva. Obstet Gynecol 1987;70:774–84.

[12] Boyce J, Fruchter RG, Kasambilides E, Nicastri AD, Sedlis A, Remy JC. Prognostic factors in carcinoma of the vulva. Gynecol Oncol 1985;20:364–77.

[13] Andreasson B, Nyboe J. Predictive factors with reference to low-risk of metastases in squamous carcinoma of the vulvar region. Gynecol Oncol 1985;21:196–206.

[14] Johnson GA, Mannel RM, Khalifa M, Walker JL, Wren M, Kyung-Whan M, et al. Epidermal growth factor receptor in vulvar malignancies and its relationship to metastasis and patient survival. Gynecol Oncol 1997; 65:425–9.

[15] Shepherd JH. Cervical and vulvar cancer: changes in FIGO definitions of staging. Br J Obstet Gynaecol 1996;103:405–6.

[16] Vulva. American Joint Committee on Cancer: AJCC cancer staging manual. 5th ed. Philadelphia: Lippincott-Raven; 1997. p. 181–4.

[17] Fu YS, Reagan JW. Pathology of the uterine cervix, vagina, and vulva. Philadelphia: Saunders; 1989. p. 169.

[18] De Hullu JA, Hollema H, Lolkema S, Boezen M, Boonstra H, Burger MP, et al. Vulvar carcinoma. The price of less radical surgery. Cancer 2002; 11:2331–8.

[19] Berchuck A, Rodriguez G, Kamel A, Soper JT, Clarke-Pearson DL, Bast Jr RC. Expression of epidermal growth factor receptor and HER-2/neu in normal and neoplastic cervix, vulva, and vagina. Obstet Gynecol 1990; 76:381–7.

[20] Pfeiffer D, Stellwag B, Pfeiffer A, Borlinghaus P, Meier W, Scheidel P. Clinical implications of the epidermal growth factor receptor in the squamous cell carcinoma of the uterine cervix. Gynecol Oncol 1989; 33:146–50.

[21] Hale RJ, Buckley CH, Gullick WJ, Fox H, Williams J, Wilcox FL. Prognostic value of epidermal growth factor receptor expression in cervical carcinoma. J Clin Pathol 1993;46:149–53.

[22] Kersemaekers AM, Fleuren GJ, Kenter GG, van den Broek LJ, Uljee SM, Hermans J, et al. Oncogene alterations in carcinomas of the uterine cervix: overexpression of the epidermal growth factor receptor is associated with poor prognosis. Clin Cancer Res 1999;5:577–86.

[23] Khalifa MA, Abdoh AA, Mannel RS, Haraway SD, Walker JL, Min KW. Prognostic utility of epidermal growth factor receptor overexpression in endometrial adenocarcinoma. Cancer 1994;73:370–6.